REMARKS

Telephone Interview

Applicants appreciate the interest in, and understanding of, the present invention shown by the Examiner during the Telephone Interview of June 14, 2010, and the generous amount of time the Examiner granted for Applicants to develop the outstanding issues and decide on a course of action.

During the Telephone Interview the Examiner explained that the claims as presented for examination recited intended results and inherent properties and did not sufficiently set forth concrete process steps.

Applicants explained that a characteristic kinetic quantity of a chemical reaction (forward and reverse reaction) can be determined by rapidly varying a thermodynamic state such as temperature or pressure, thereby changing the equilibrium position of the chemical reaction, and monitoring the shape and speed by which the chemical species involved in the reaction relax into the new equilibrium state.

Thus, the equations for computing chemical reaction rates are well known; however, there were problems due to technical steps required to bring about the rapid changes in physical state, and problems due to the damage done to sensitive chemical species by the rapid varying of temperature or pressure.

The present invention is based on the discovery that relaxation kinetics can be measured - without the need to introduce rapid temperature or pressure shift, and without the attendant damage to sensitive chemical species – by using light to generate a non-equilibrium state of the chemical reaction, and then observing, by means of the fluorescence signal of at least one fluorophore, at least a portion of the relaxation of concentrations of the species involved.

As explained in paragraph [0016] of the specification as published, "the rate constants for the photochromic process of switching off the free ligand A (k_{+-}) and the complex DA (k'_{+-}), respectively, are different from each other. The reason for this is a FRET channel of excitation which makes the process of switching off more efficient for the complex than for the free ligand

($k'_{+-}>k_{+-}$)." As explained in paragraphs [0040] and [0041], "Since there is a high FRET efficiency due to the ON state of the FRET acceptor (A_+), an asymmetry of the total system results yielding an underpopulation of the state DA_+ of the bound FRET pair compared to the population of the state A_+ of the free FRET acceptor. This asymmetry leads to the inequality of the rate constants k_{+-} and k'_{+-} mentioned earlier with reference of to the reaction equation (1), i.e. to the different impacts of the irradiation on the left side and on the right side of the equation of the example reaction. It is obvious that in this way a non-equilibrium of concentrations is created with a thermodynamically unchanged position of the equilibrium."

As a result of the Telephone Interview, Applicants have drafted new claims 17 and 18 to better set forth the invention in accordance with US claim practice.

Applicants are in continuing consultation with the inventors so that the claims can be further revised if necessary, and respectfully request that the Examiner not act on the present Amendment for at least one month so that Applicants can finalize the base claim.

Status of Claims

Claims 1-13 were previously pending.

Claims 14-16 were cancelled.

New claims 17 and 18 are added setting forth more precise sequential process steps based on formula (1)-(3) and associated text, and in particular paragraphs [0015], [0016] , [0040] and [0041].

Response to Arguments

Regarding the rejection of claims 1-13 under 35 U.S.C. 112, first paragraph, scope of enablement, Applicants argue that the instant amendment overcomes the rejection, because the non- equilibrium concentration refers not to the concentration of reactants in a chemical reaction but to the concentration of switched acceptor molecules. However, the active steps of the amended claim 1 read as follows:

"having light of a wavelength capable of switching said photochromic state of said FRET acceptor impinge on said sample with the chemical reaction being in its equilibrium state, thereby switching said photochromic state of said acceptor in said product of said chemical reaction less efficiently than in said free ligand, thus generating a non-equilibrium state of said chemical reaction,

observing, by means of a FRET dependent fluorescence signal of at least one of said fluorophore and said acceptor at least one temporal portion of a relaxation of concentrations of said species involved" (emphasis added).

Therefore, contrary to Applicants' assertion, the claims still read on creating a non-equilibrium state of the chemical reaction by light excitation and observation of the concentration of the reactants, rather than creating the non-equilibrium concentration of the excited acceptor molecules and observing the relaxation of the acceptor molecules.
The rejection is maintained.

In response, Applicants submit that the claims as amended clearly require and set forth the creation of a non-equilibrium state, followed by observation of relaxation.

Withdrawal of the rejection is respectfully requested.

Claim Interpretation

The term "characteristic kinetic quantity of a chemical reaction" has not been defined by Applicants, therefore it is interpreted as any measurable variable.

In response, Applicants submit new claims 17 and 18 and respectfully request examination of these claims.

Claim Rejections - 35 USC § 112

Claims 1-13 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for monitoring chemical reactions in which the chemical species are fluorophores themselves and in which the physical or chemical properties of the fluorophores are

changed upon irradiation with light in such a way as to create populations of molecules in two different states where the populations of molecules are different from the populations before the irradiation, does not reasonably provide enablement for monitoring chemical reactions with any molecule having a fluorophore attached to it in any other chemical reaction. In addition, there is no enablement for determining any kinetic quantity of any chemical reaction. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

In response, Applicants have crafted new claims 17 and 18 which are believed to overcome the rejections under this paragraph.

Withdrawal of the rejection is respectfully requested.

Claim Rejections - 35 USC § 103

Claims 1-3 and 7-13 are rejected under 35 U.S.C. 103(a) as being unpatentable over Gadella et al. (J. Cell Biol., vol. 129, pp. 1543-1558, 1995) and Song et al. (J. Photochem. Photobiol., vol. 150, pp. 177-185, July '26, 2002).

According to the Examiner, regarding claim 1, Gadella et al. teach detection of oligomerization of the epidermal growth factor (EGF) molecules in a reaction involving free EGF molecules labeled with fluorophores (Abstract):

wherein said chemical reaction involves a plurality of chemical species, at least a first one of said species including one a fluorophore being a FRET acceptor of a FRET pair consisting of a FRET donor and a FRET acceptor and a at least a second one of said species including a fluorophore being a FRET donor of said FRET pair (page 1545, second paragraph, where the first species is EGF labeled with fluorescein (donor) and the second species is EGF labeled with tetramethyl-rhodamine (acceptor),) said donor being a fluorofore, the emission spectrum of which having an overlap region with said FRET acceptor's absorption spectrum (page 1544, last paragraph),

wherein said chemical reaction reversibly converts said first and second species as free ligands into at least one product comprising a combination of said first and second species (page

1545, fourth paragraph; page 1548, fourth and fifth paragraph; page 1549, first and second paragraph; page 1554, third paragraph; where the free EGF molecules are converted into a dimmer), the method comprising the steps of:

having light of a wavelength capable of switching said photochromic state of said FRET acceptor impinge on said sample with the chemical reaction being in its equilibrium state (page 1545, fifth and sixth paragraph; page 1548, paragraphs 3-5; Fig. 3-5), and

observing, by means of a FRET dependent fluorescence signal of at least one of said fluorophore and said acceptor at least one temporal portion of a relaxation of concentrations of said species involved (page 1545, fifth and sixth paragraph; page 1548, paragraphs 3-5; Fig. 3-5).

Regarding claim 2, Gadella et al. teach detecting fluorescence of the donor (page 1545, sixth paragraph; page 1548, third paragraph).

Regarding claim 10, Gadella et al. teach excitation of FRET acceptor with visible light (page 1545, fifth paragraph).

Gadella et al. do not teach the acceptor being a photochromic molecule or observing FRET effect between switched states of the photochromic acceptor and a fluorescent donor.

Regarding claim 1, Song et al. teach a photochromioc acceptor molecule which can act as a light-induced switch in FRET (Abstract; page 178, third and last paragraph; page 179-181; page 182, paragraphs 1-3).

Regarding claim 3, Song et al. teach measuring fluorescence of the photochromic acceptor (page 179, third paragraph; page 180, fourth paragraph; Fig. 2).
Regarding claims 7-10, Song et al. teach switching the photochromic acceptor between the ON and OFF states using two different wavelengths, one of them being in the UV region and the other in the visible region (page 178, third paragraph; page 179, third paragraph; Fig. 2).

Regarding claim 11, Song et al. teach the intensity of irradiation for the photochromic effect being higher than the intensity of irradiation to observe the fluorescence (page 179, third and fourth paragraphs).

Regarding claims 12 and 13, Song et al. teach irradiating the sample in an alternating (—temporarily modulated) fashion to change the photochromic effect of the acceptor (page 182, last two paragraphs; Fig. 5; page 183, first paragraph).

It would have been prima facie obvious to one of ordinary skill in the art at the time of the invention to have used the photochromic acceptor of Song et al. in the method of FRET microscopy of Gadella et al. The motivation to do so is expressly provided by Song et al., who state (page 178, second and third paragraphs; page 184, last paragraph):

"For static systems under cellular fixation, the pbFRET techniques are simple and useful [28-31]. However, photobleaching rates are linear or more complex [21] functions of irradiance and dependent on environmental parameters such as the local redox state [32,33]. In addition the photodestruction of donor or acceptor precludes the possibility of multiple FRET measurements at a given spatial location.

Our aim was to design a method that offers an intrinsic internal reference and the possibility for continued and repeated determinations of the FRET process. Photochrotnic compounds can be exploited to achieve this goal. Photochromism is the light-induced transformation of a single chemical species between two isomeric structures with distinct absorption spectra [34-37]. Near- ultraviolet irradiation induces reversible changes in the structure and absorption properties of a photochromic molecule from an initial colorless to a colored form. Only the latter has an absorption band overlapping the emission band of a selected donor and is thereby able to potentiate energy transfer. Subsequent irradiation in the visible absorption band of the colored form reverts it to the initial colorless form and disables the FRET process, thus supplying the required internal reference state. The FRET efficiency can be derived simply from the fractional change in donor fluorescence or donor lifetime for each pixel position."

"The photoreversibility of the photochromic acceptor offers a unique opportunity for performing repeated quantitative FRET observations under the unknown stoichiometry of the donor—acceptor relationships pre-valuing miciu3copy e pei;iiients. A hotochromic acceptor

offers the critical internal control (acceptor-free donor) within the same sample preparation and at every spatial location and chemical environment."

Claims 4-6 are rejected under 35 U.S.C. 103(a) as being unpatentable over Gadella et al. (J. Cell Biol., vol. 129, pp. 1543-1558, 1995) and Song et al. (J. Photochem. Photobiol., vol. 150, pp. 177-185, July 26, 2002), as applied to claim 1 above, and further in view of Watrob et al. (J. Am. Chem. Soc., vol. 125, pp. 7336-7343, May 2003; cited in the previous office action). Regarding claims 4-6, Giordano et al. teach a system with one acceptor and one donor, but do not teach a system with an additional acceptor.
Regarding claims 4-6, Watrob et al. teach using a system with one donor and two acceptors (Scheme 1; page 7337, 7338).

It would have been prima facie obvious to one of ordinary skill in the art at the time of the invention to have used additional acceptor of Watrob et al. in the method of Gadella et al. and Song et al. The motivation to do so is provided by Watrob et al., who state (page 7342, last paragraph):

"Three-chromophore FRET systems offer several advantages. First, three-chromophore systems report the simultaneous proximity of three species and provide the ability to measure two or three distances in a complex. Structural information about the assembly can then be inferred from the relative positions of individual components of the complex. For example, in Case I where no FRET1 -> 3 occurs, r13 must be >1.5 R013. This restricts the position of 3 relative to 1 to a minimal distance of r13 — 1.75 R013 and a maximal distance of r13 = r12 + r23 for a linear arrangement of 1, 2, and 3. Second, in the case of linear or near linear arrangement of the three chromophores, two-step FRET extends the distance range for detection of simultaneous proximity-. For example, assuming R0 = 55 A for the two FRET pairs and a detection limit of 1.5 R0, one-step FRET at a distance r = 83 A has an efficiency Eij = 0.08. A two-step FRET relay with Erelay = 0.08 corresponds to a total distance r = 127 A. Thus, the detectable distance range increases by as much as 50%. Third, threechromophore systems require fewer labeled samples to measure two or three distances than conventional one-step FRET.".

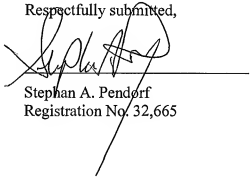
Applicants respectfully traverse, in particular with respect to new claims 17 and 18.

Accordingly, withdrawal of the rejection is respectfully requested.

The Commissioner is hereby authorized to charge any fees which may be required at any time during the prosecution of this application without specific authorization, or credit any overpayment, to Deposit Account Number 16-0877.

Favorable consideration and early issuance of the Notice of Allowance are respectfully requested. **Should further issues remain prior to allowance, the Examiner is respectfully requested to contact the undersigned at the indicated telephone number.**

Respectfully submitted,



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Date: **June 16, 2010**